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STUDIES ON VISCERAL REGENERATION IN SEA-STARS. II. REGENERATION OF PYLORIC CAECA IN ASTERIIDAE, WITH NOTES ON THE SOURCE OF CELLS IN REGENERATING ORGANS [1]

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In a previous article (Anderson, 1962) it was shown that sea-stars from which pyloric caeca had been operatively removed were capable of making good progress towards the eventual replacement of these organs within the relatively brief period of time (8 weeks) covered by the experiments reported. These earlier studies demonstrated that in *Henricia leviuscula* (Family Echinasteridae) the preliminary steps in regeneration of the caecum produced a cylindrical, tubular outgrowth advancing between suspending mesenteries from the proximal stumps of the severed pyloric ducts. Presumably the Tiedemann's pouches, flagellary pumping organs which are normal appendages of the oral sides of the caeca, are subsequently elaborated by modification of the floor of the tubular outgrowth. Histological study of the regenerating organs showed a clear distal-proximal gradient of differentiation, with no trace of the precociously differentiated tip characteristic of the parietal parts of a regenerating ray. As no zone of cellular proliferation could be identified in the *Henricia* material, it was suggested that outgrowth of the regenerating caecum might involve the mobilization and incorporation of amoebocytes wandering in the connective tissues and fluid spaces of the body, reaching the regenerate by way of the mesenteric sheets always reconstituted in advance of the outgrowing tube.

Experiments similar to those already reported for *Henricia* have also been performed on three species (*Asterias forbesi*, *Pisaster ochraceus*, and *Leptasterias pusilla*) belonging to the Family Asteriidae. In these sea-stars the pyloric caeca are relatively simple, lacking Tiedemann's pouches, and the replacement of a functional organ after extirpation might be expected to proceed more rapidly and in less complicated fashion than in *Henricia*. It is my intention to present here a relatively brief, composite account of the results of my studies on these three species,

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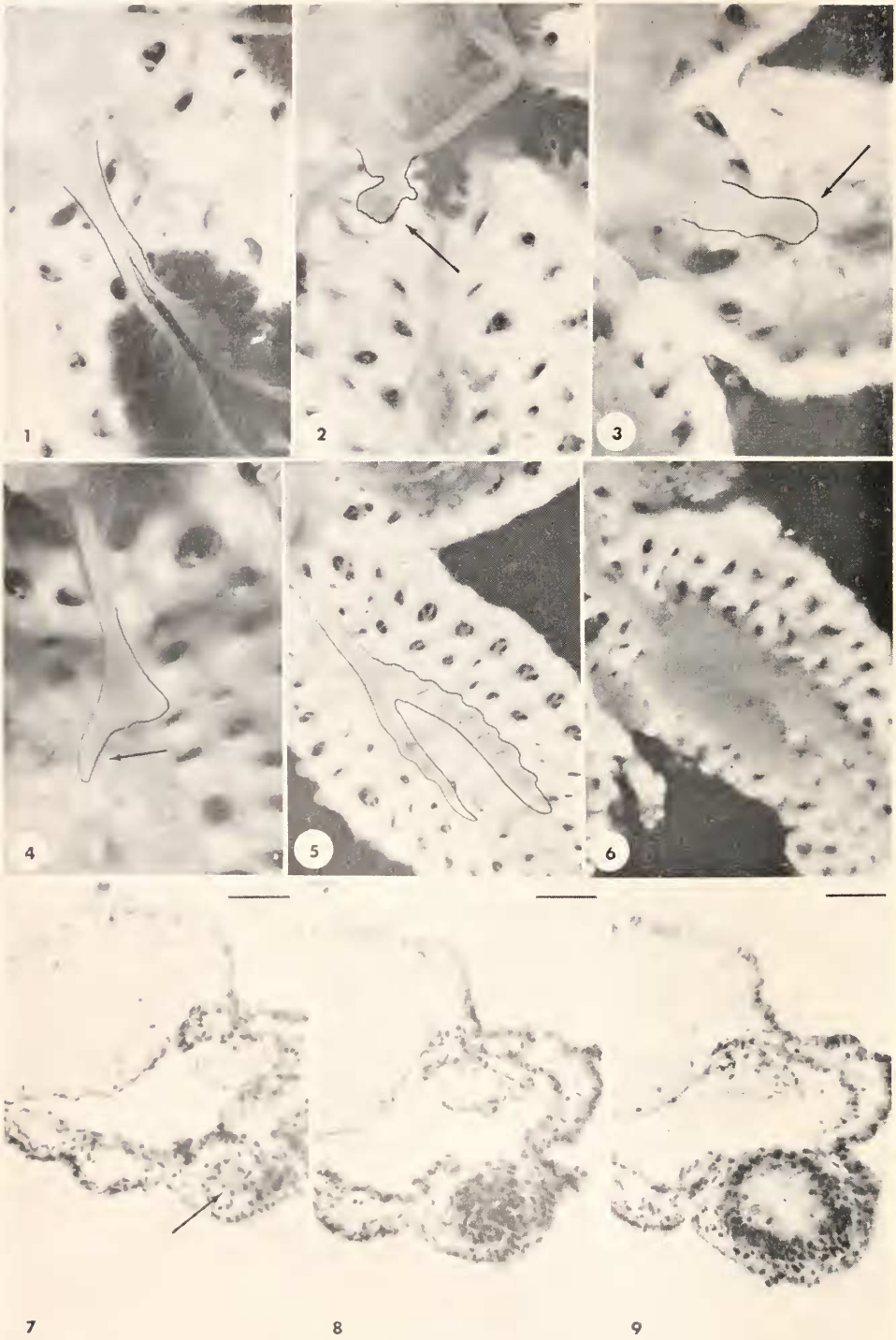


FIGURES 1–9.

with particular attention to points of comparative interest in relation to the earlier studies on *Henricia* and to points not brought out in those studies.

A further series of experiments involved the administration of tritiated thymidine to specimens of *Asterias forbesi* at a known stage in the regeneration of pyloric caeca only, or in the replacement of entire autotomized rays. Autoradiographs prepared from tissues of these animals have provided new information on the production of cells making up the regenerating parts, and the results of these experiments will be presented.

The work on the West Coast species was carried on at the Hopkins Marine Station of Stanford University, Pacific Grove, California. Once again, I record my sincere appreciation of the many kindnesses extended by the Director and staff of this station. It is a pleasure to acknowledge also the competent technical assistance of Rebecca Folsom Ferguson in experiments carried out at the Marine Biological Laboratory, Woods Hole.

Materials and Methods

Small specimens of *Leptasterias pusilla*, radius about 3 cm. or less, were collected intertidally at Mussel Point and Point Piños, in Monterey Bay; *Pisaster*

Figure 1. *Asterias:* single pyloric duct leading from the pyloric stomach (upper left) and branching distally to form the paired pyloric caeca, characteristic of members of the Family Asteriidae. In operative removal of the pyloric caeca this duct is severed near its origin from the pyloric stomach. (Approx. 6 ×.)

Figure 2. *Asterias:* stump of severed pyloric duct (arrow) one week after removal of the pyloric caeca. The diffuse dark area below marks the site of the incision in the aboral body wall and shows the extent of post-operative healing in 7 days. (Approx. 6 ×.)

Figure 3. *Asterias:* pyloric duct (arrow) 10 days after removal of the pyloric caeca. (Approx. 8 ×.)

Figure 4. *Asterias:* appearance of the regenerating duct two weeks after removal of the pyloric caeca; this shows the incipient bifurcation of the regenerate. Arrow indicates the approximate level of the cross-section shown in Figure 26. (Approx. 12 ×.)

Figure 5. *Asterias:* progress in regeneration three weeks after removal of the pyloric caeca. Note that one of the branches is longer and broader than the other and shows developing folds in its wall. The sections shown in Figures 7 through 11 were made from this specimen. (Approx. 3 ×.)

Figure 6. *Asterias:* regenerated pyloric caeca 5 weeks after removal of the original pair. Note the extensive folding and branching of the walls of both replacement organs; from all indications the regenerate is now fully functional. (Approx. 3 ×.)

Figures 7 through 11 represent cross-sections of regenerating pyloric caeca in the three-week specimen shown in Figure 5. Tissue fixed in Helly's fluid, sections stained in phosphotungstic acid hematoxylin.

Figure 7. *Asterias:* section just distal to the end of the shorter, smaller regenerating tube. The mesenteric sheets hanging from the aboral body wall have fused to form a continuous tunnel; the summit of the tunnel at this level contains an accumulation of mesenchyme cells (arrow). (Scale = 25 μ.)

Figure 8. *Asterias:* similar section of the same regenerate just proximal to the last. The dark mass of cells occupying the summit of the tunnel represents the closed end of the tubular outgrowth advancing between the mesenteric sheets. (Scale = 25 μ.)

Figure 9. *Asterias:* the same regenerate sectioned somewhat more proximally. The lumen is well developed even at this far distal level, and its lining epithelium consists of tall cells with flagella and a brush border, with nuclei crowded toward the base. (Scale = 25 μ.)

specimens of about the same size were obtained at Mussel Point and along the shore just north of Santa Cruz, in an area where small individuals are locally abundant. These animals were kept in running sea-water at the Hopkins Station and were fed occasionally on cracked snails and pieces of mussel flesh. Similarly small specimens of *Asterias forbesi* were provided by the Supply Department at Woods Hole and maintained in the circulating sea-water of the Laboratory; these animals were also fed on small snails and bivalves.

In most cases, operative technics for this series of experiments were as previously described (Anderson, 1962) for the studies on *Henricia*. Animals were immobilized by soaking in $MgCl_2$ solution and the paired pyloric caeca removed from one ray through a longitudinal incision in the aboral body wall. Removal of the caeca involved transecting the pyloric duct (which in these species, unlike *Henricia*, is single as it leaves the pyloric stomach; see Figure 1) and cutting or tearing the mesenteries attaching the caeca to the body wall. In a few instances, to determine the effects of partial extirpation of the organs, only one member of the pair was removed from a ray, or only intermediate or terminal portions of a member. In other cases, to check the animals' ability to repair more massive defects, the pyloric caeca were removed from all the rays. Returned to running sea-water, the animals usually recovered rapidly, and the operative incisions healed well without the use of medication or sutures.

To follow the course of regeneration, individuals were sacrificed and examined at approximately weekly intervals, beginning at the close of the first post-operative week. Observations were continued for 12 weeks on *Pisaster*, for 10½ weeks on *Leptasterias*, and for 6 weeks on *Asterias;* the extended duration of the experiments with the West Coast forms reflects the relatively slower rate of regeneration found to be characteristic of these species. Some individuals from which all of the pyloric caeca had been removed were followed for longer periods; in the case of one *Pisaster*, observations were continued for 16 weeks. These animals were checked periodically with respect to feeding behavior and ability to digest material that had been ingested.

Procedures for determining the progress of regeneration were as previously outlined and will not be described in detail here. As before, fixation of tissues was in Helly's fluid, chosen particularly in order to preserve cytoplasmic elements of the secretory cells in the regenerating organs. Decalcification involved soaking the tissues for about a week in 5% aqueous disodium EDTA, a chelating agent. Paraffin sections of the aboral body wall with attached regenerating viscera were cut at 7 to 10 μ and stained in Mallory's phosphotungstic acid hematoxylin.

For the experiments on *Asterias* utilizing tritiated thymidine, groups of animals were prepared by removing one pair of pyloric caeca from each by the standard technic. After these animals had regenerated for two weeks each received, in the body cavity of the operated ray or an adjacent ray, an injection of either 0.05 or 0.10 ml. of tritiated thymidine solution. The solutions were made up in sterile sea-water in concentrations calculated to provide 1.0, 10.0, or 100.0 μc./ml.; the total activity administered to each animal thus varied between 0.05 and 10.0 μc. Injected animals were kept separately in small bowls of cool sea-water for 3, 6, 12, or 24 hours. For comparison, four additional individuals were used which had been regenerating entire rays, not just excised pyloric caeca, for two weeks following

induced autotomy. These received comparable injections of the same solutions and were maintained for 6 or 12 hours.

Following the indicated period of incubation the animals regenerating pyloric caeca were relaxed in $MgCl_2$ and carefully dissected and the extent of regeneration noted. The aboral body wall of the operated ray, bearing the regenerating parts, was pinned out on wax and flooded with Bouin's fluid. After a brief hardening period the tissue was trimmed and transferred to a vial of the same fixative and placed under partial vacuum in a desiccator. Bouin's fluid was chosen because, in addition to its efficacy in preserving nuclear details, its acidity brings about rapid decalcification of the body-wall ossicles and obviates the necessity of soaking the tissue for long periods in the customary EDTA solutions, with attendant loss of radioactive compounds. The partial vacuum promoted the escape of gas bubbles resulting from decalcification and minimized tissue distortion. After one to two days' fixation and decalcification the tissues were washed in 80% alcohol, dehydrated, embedded in paraffin by standard technics, and sectioned at 10 μ. Animals regenerating entire rays were handled similarly except that they were not dissected before fixation.

Alternate slides from the sets bearing sections of these tissues were used in the preparation of autoradiographs. They were dipped in Kodak nuclear track emulsion (Type NTB-3), air-dried, sealed in light-tight boxes with a desiccant, and kept in a freezer for 7 days. They were then developed in D-19 and stained in dilute Harris' hematoxylin. The remaining slides were routinely stained in Mallory's phosphotungstic acid hematoxylin, for purposes of comparison and to reveal cytological details obscured by the stained emulsion overlying the sections in the autoradiograph slides.

Results

For any one of the species studied here, the sequence of events in caecal regeneration, while not entirely uniform, is consistent and predictable within limits. For example, *Asterias forbesi,* under the conditions of these experiments, can replace a set of excised pyloric caeca with a reasonably complete regenerate within 5 to 6 weeks. The first postoperative week brings little grossly observable progress (Fig. 2) beyond closure and partial healing of the body-wall incision. By 10 days (Fig. 3) a blind tubular outgrowth appears at the end of the severed pyloric duct, and usually within two weeks this outgrowth has at least begun the process of bifurcation which is responsible for the pairing of the replacement organ. The two branches thus formed continue to elongate during the next couple of weeks, one usually extending somewhat farther than the other. At the same time (Fig. 5) they become broader and deeper and develop folds and incipient sacculations in their side walls. By 5 weeks (Fig. 6) this process has produced the complex, branching side pockets characteristic of the complete organ; the caeca at this time are still somewhat smaller than those in adjacent intact rays, but another week or two of continued growth will undoubtedly bring them to their normal size.

Pisaster and *Leptasterias* evidently replace extirpated caeca through the same processes observed in *Asterias,* as structurally similar stages can be found in all three species. In the Californian forms, however, the rate of regeneration is considerably slower. Figure 12, for instance, shows a *Pisaster* regenerate typical

of a series of 8-week specimens examined; comparison with Figure 5 makes it evident that *Asterias* has made more progress in three weeks than *Pisaster* made in 8. The nearly complete pair of replacement caeca shown in Figure 14 for *Pisaster* is structurally about equivalent to the *Asterias* regenerate pictured in Figure 6; it will be noted, however, that this represents a 5-week regenerate in *Asterias* but a 12-week growth in *Pisaster*. The rate in *Leptasterias* is closer to that in *Pisaster* than to *Asterias;* the beginning of bifurcation is not seen until four weeks. The 5- and 6-week regenerates shown in Figures 16 and 17, while in some respects better developed than an 8-week *Pisaster* (Fig. 12), are much simpler than the three-week *Asterias* caeca, with their walls already somewhat folded, seen in Figure 5. The oldest *Leptasterias* regenerate in the series, the 10½-week specimen in Figure 18, does not approach the structural complexity of the 12-week *Pisaster* (Fig. 14) or the 5-week *Asterias* (Fig. 6).

Rate differences aside, however, histological studies show that at equivalent morphological stages, irrespective of age, regenerates of the three species under investigation are very similar to one another and are obviously formed through similar processes. Therefore, in a general description of the events in regeneration and differentiation, illustrative examples drawn from any of the species should be broadly applicable to all. Further, in any regenerate, of whatever age, the youngest, simplest, most recently added part lies at the distal tip and is followed in a proximal gradient by regions successively older, more mature, and more complex. It should be borne in mind that the growing tip of an advanced regenerate is histologically equivalent to the corresponding region in any younger regenerate, and that following serial sections of a single specimen from tip to base affords an opportunity of studying, in what amounts to a time-sequence, the processes of growth and differentiation in the developing organ.

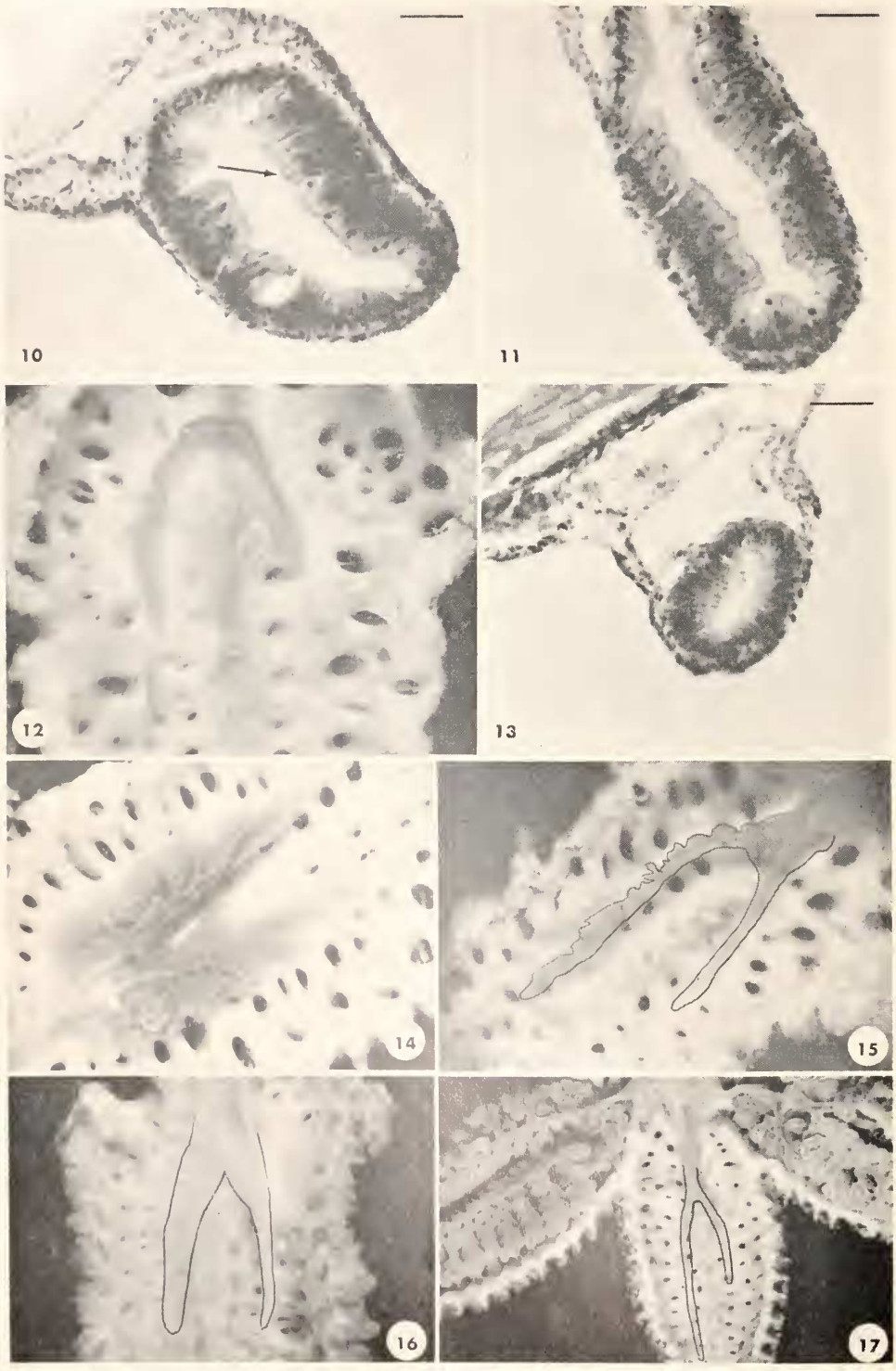
After the repair of the operative incision, the earliest internal events in caecum regeneration involve fusion of the torn edges of the mesenteries to form a continuous tunnel. This consists of double mesothelial sheets separated by a thin connective-tissue and mesenchymal layer. There is no general hypertrophy of the peritoneum, as previously observed in *Henricia* (Anderson, 1962); in *Leptasterias* the mesothelial cells immediately overlying the regenerate appear unusually tall and basophilic (Figs. 19, 20), but this is not at all comparable to the widespread involvement of the peritoneum seen in *Henricia*. Under ideal circumstances the course of the mesenteric tunnel corresponds precisely to the lines of attachment of the original caeca, and thus the tunnel forks at the point of bifurcation of the original organ and continues into the ray as a pair of parallel structures, one on either side of the healing incision. Distally these are low, flat ridges, but proximal to the bifurcation they become more elevated and join the mesenteries supporting the healed-over stump of the pyloric duct. As in *Henricia*, the preliminary formation of these tunnels provides a guide for the subsequent outgrowth of the regenerating caecum. Occasional cases of deranged regeneration can often be explained as resulting from unusually severe damage to the original mesenteries, and to the peritoneum with which they are continuous, during the operation in which the pyloric caeca were excised.

With at least the proximal portions of the guiding mesenteries reconstituted, replacement of the caecum itself begins. Sections show that the outgrowth observed

in the dissections consists of a cellular tube, continuous with the lining of the pyloric duct, advancing through the mesenchymal layer between the mesothelial sheets at the summit of the tunnel. As this outgrowth reaches the bifurcation of the tunnel, it broadens, and then from its two outer corners a pair of smaller tubes appears and invades the parallel tunnels leading distally into the ray. Since the distal portions of regenerates of any age present the same histological features, the nature of these very young outgrowths can be understood by reference to sections of older ones. Immediately in advance of the growing tip there appears an accumulation of mesenchyme cells, as shown in Figure 7. The outgrowth itself comprises a solid mass of cells only at its extreme end (Figs. 8, 19); within a very short distance proximally a lumen appears, continuous with that of the pyloric duct (Figs. 9, 13, 20). Very close behind the tip the cells lining the lumen show many of the characteristics of those found in the epithelium of the mature organ; they are relatively tall, with nuclei crowded toward the basal end and with flagella and a brush border at the free end, as seen in Figure 9. Further, even very near the end of the tube, and in regenerates of only 10 days' growth, the epithelium contains secretory elements like those found in the normal caecum; zymogen cells (Fig. 13) and mucous goblets (Fig. 20) appear in the epithelium almost as far out as the lumen extends.

As the tip of the tube progresses distally, it thus establishes and leaves behind at more proximal levels the basic cell groups out of which the mature regenerate develops. Figures 10 and 11, representing sections some distance back from the tips of the two regenerating tubes seen in Figure 5, show the characteristics and relationships of the cell layers making up the forming organ. In the tall columnar epithelium lining the lumen, both zymogen and mucous gland cells are present in increased numbers as compared with more distal areas. The mesothelia covering the outer surfaces of the regenerate have become stretched and flattened as the diameters of the epithelial tubes have increased and their proportions have altered. Between the lining epithelium and the covering peritoneum, what appear to be remnants of the mesenchymal accumulations in the tunnel are spread in a thin layer, forming the probable antecedents of the subepithelial connective-tissue and muscle components of the developing organ.

Figures 21 and 22 show, respectively, sections of distal and proximal levels of the *Leptasterias* regenerate seen in Figure 16. The thin-walled, cavernous nature of the tubes at these levels is evident, but it will be noted that in Figure 22, at the point of bifurcation of the regenerate, localized thickenings have produced inward bulges in the lining, representing the beginnings of the folds and pouches characteristic of the mature organ. Figure 23, showing sections through the branches of a much older and better-developed regenerate, illustrates a further stage in the pouching process as well as a second very significant change. This involves localization of differentiated cell types in specific areas of the lining, as a result of which the side-walls, forming the precursors of the glandular pouches of the caecum, come to contain practically all of the secretory elements of the epithelium. In contrast, the roof and floor of the regenerate, which will constitute the median duct of the caecum, are lined with relatively unspecialized flagellated cells engaged in current production and responsible for maintenance of the characteristic pattern of circulation in the contents of the organ. That this circulation has already been set



FIGURES 10–17.

up even in very immature distal regions of a regenerate is indicated by Figure 13, which shows that debris characteristic of the external environment has been carried to the extreme end of the lumen of this regenerating tube.

There can be little doubt that the functional capacities of the regenerate are established early and progressively develop towards normalcy as the size and structural complexity of the organ continue to increase, as secretory elements differentiate in increasing abundance and become localized in the glandular pockets, as the normal patterns of movement and circulation of the fluid contents assert themselves, and as the subepithelial muscular, connective-tissue, and nervous elements develop in the wall of the caecum. It is evident that from relatively early stages the regenerate functions as an organ, even though in terms of structural and functional differentiation its immature distal extremities lag far behind the older proximal portions.

For comparison with the general process of regeneration which can now be taken as established, a smaller series of experiments involving either partial or total caecal extirpation provides instructive material. Figure 24, for example, illustrates the result of an experiment in which a single member of the pair of caeca in one ray was excised, 6 weeks previously, in *Leptasterias*. Comparison with Figure 17 makes it apparent that the presence of the intact caecum in the ray has had no demonstrable effect on the rate of replacement of the excised member. The caecal tube has clearly grown out from the point of amputation of the original, following the path established by the mesenteric attachments of the missing portion. It is probably only coincidental that in the 6-week period provided, the regenerate has extended to a point almost exactly level with the tip of the normal member. Histologically, the tubular organ at this stage is in all respects comparable with

FIGURE 10. *Asterias:* the three-week regenerate at a still more proximal level. The tube here is taller than broad, and in addition to the conspicuous mucous goblet, the epithelium contains numerous zymogen cells lying in the region indicated by the arrow. Note also the large nuclei scattered high in the epithelium. (Scale = 25 μ.)

FIGURE 11. *Asterias:* cross-section of the larger of the two regenerating tubes shown in Figure 5. The epithelium contains numbers of zymogen cells and mucous goblets; except for the presence of the unusually numerous large nuclei in the clear areas bordering on the lumen, it appears very similar to the normal caecal epithelium. (Scale = 25 μ.)

FIGURE 12. *Pisaster:* specimen dissected after 8 weeks' regeneration following removal of pyloric caeca. The forked regenerate extends distally from the single pyloric duct at top center. Each fork is a deep tube with walls only weakly folded. (Approx. 6 ×.)

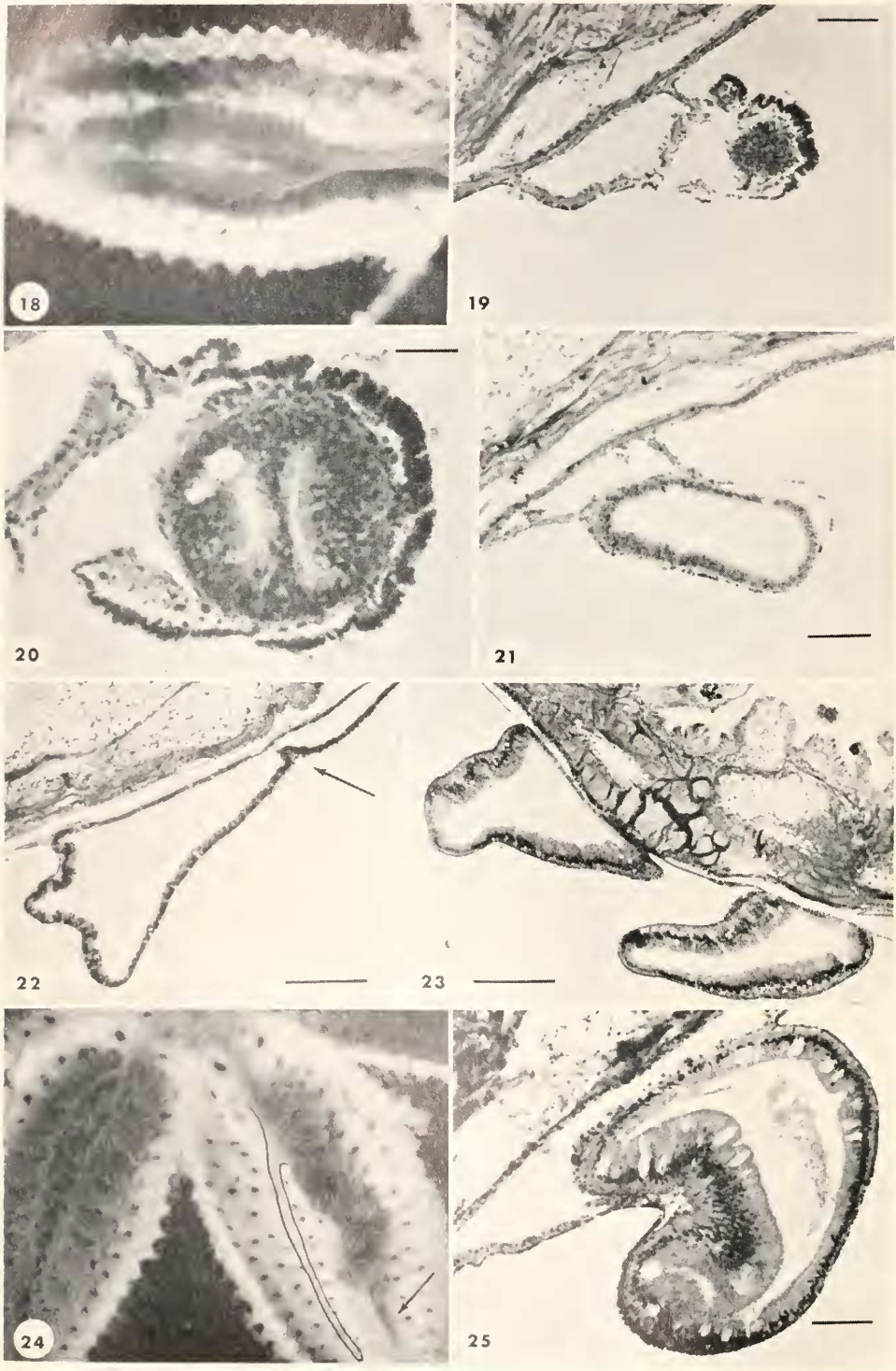
FIGURE 13. *Pisaster:* cross-section of an 11-week regenerate, far distally. As in *Asterias* (*cf.* Fig. 9), the regenerate advances between mesenteric sheets in a guiding tunnel. The lining epithelium shows differentiated secretory cells, as well as flagella and brush border, and the lumen contains detritus from the external environment. PTA hematoxylin. (Scale = 25 μ.)

FIGURE 14. *Pisaster:* well-developed regenerate 12 weeks after removal of the original pair of caeca. Structurally, this is perhaps slightly more advanced than the 5-week *Asterias* regenerate shown in Figure 6. (Approx. 4 ×.)

FIGURE 15. *Pisaster:* one pair of regenerating caeca in an animal from which all 5 pairs had been removed almost 12 weeks previously. One member of this pair shows developing folds and wrinkles, but comparison with Figure 14 shows that the replacement of 5 pairs of caeca is slower than the regeneration of a single pair in the same species. (Approx. 5 ×.)

FIGURE 16. *Leptasterias:* dissection of a 5-week regenerate. The sections shown in Figures 19 through 22 were made from this specimen. (Approx. 8 ×.)

FIGURE 17. *Leptasterias:* progress in regeneration 6 weeks after removal of the pyloric caeca. As in most cases, one branch extends beyond the other. The size and relationships of the intact pyloric caeca can be seen in the adjacent unoperated rays. (Approx. 4 ×.)



FIGURES 18–25.

either of the paired regenerates of the same age shown in Figure 17. Conversely, the presence of the regenerating caecum has exerted no demonstrable effect on the normal member of the pair. Although in Figure 24 the bulk of this organ appears somewhat less than that of a normal caecum in the adjacent ray, this probably results from a difference in the way in which the organ lies in the opened coelomic cavity; approximately the same number of glandular pouches is present in all. Obviously, removal of one member of the pair has provided the remaining member additional space in which to lie. As Figure 25 shows, the extreme distal portion of the intact organ presents normal histological characteristics and shows no indications of stimulated growth or of de-differentiation, as might perhaps have been expected.

A different sort of experiment, not illustrated in the figures, provides additional information bearing on questions of growth-gradients in the regenerating system. In *Leptasterias,* one ray was opened and the proximal portion of one member of its pair of caeca was excised, leaving intact a 1-cm. length of its distal end. At the same time, the proximal and distal portions of the other member were removed, leaving the middle part in place. Opened again after a 5-week period, the animal showed significant changes. Both of the isolated portions of the two caeca, which had been left attached only by their respective mesenteries, had undergone considerable reduction and resorption, although remaining identifiable and histologically recognizable. Neither had produced regenerative outgrowths in either direction. But while these isolated distal segments had not themselves contributed any new growth, neither had they exerted any inhibitory effects on normal events in regeneration; the usual mesenteric tunnels had formed proximally

FIGURE 18. *Leptasterias:* well-developed regenerate, with simple sacculate foldings in its walls, 10½ weeks post-operative. The section shown in Figure 23 was made from this specimen. (Approx. 6 ×.)

FIGURE 19. *Leptasterias:* cross-section at the extreme end of the smaller branch of the five-week regenerate shown in Figure 16. The crowded cells occupying the summit of the tunnel mark the closed end of the advancing tube. This section is comparable in all respects with that shown in Figure 8, for *Asterias,* with the exception of the characteristic hypertrophy of the peritoneum over the regenerate in *Leptasterias.* Helly-fixed; PTA hematoxylin. (Scale = 50 μ.)

FIGURE 20. *Leptasterias:* section just proximal to the last. The lumen extends almost to the distal end of the regenerate, and even at this level the epithelium is well differentiated, containing conspicuous mucous goblets. PTA hematoxylin. (Scale = 25 μ.)

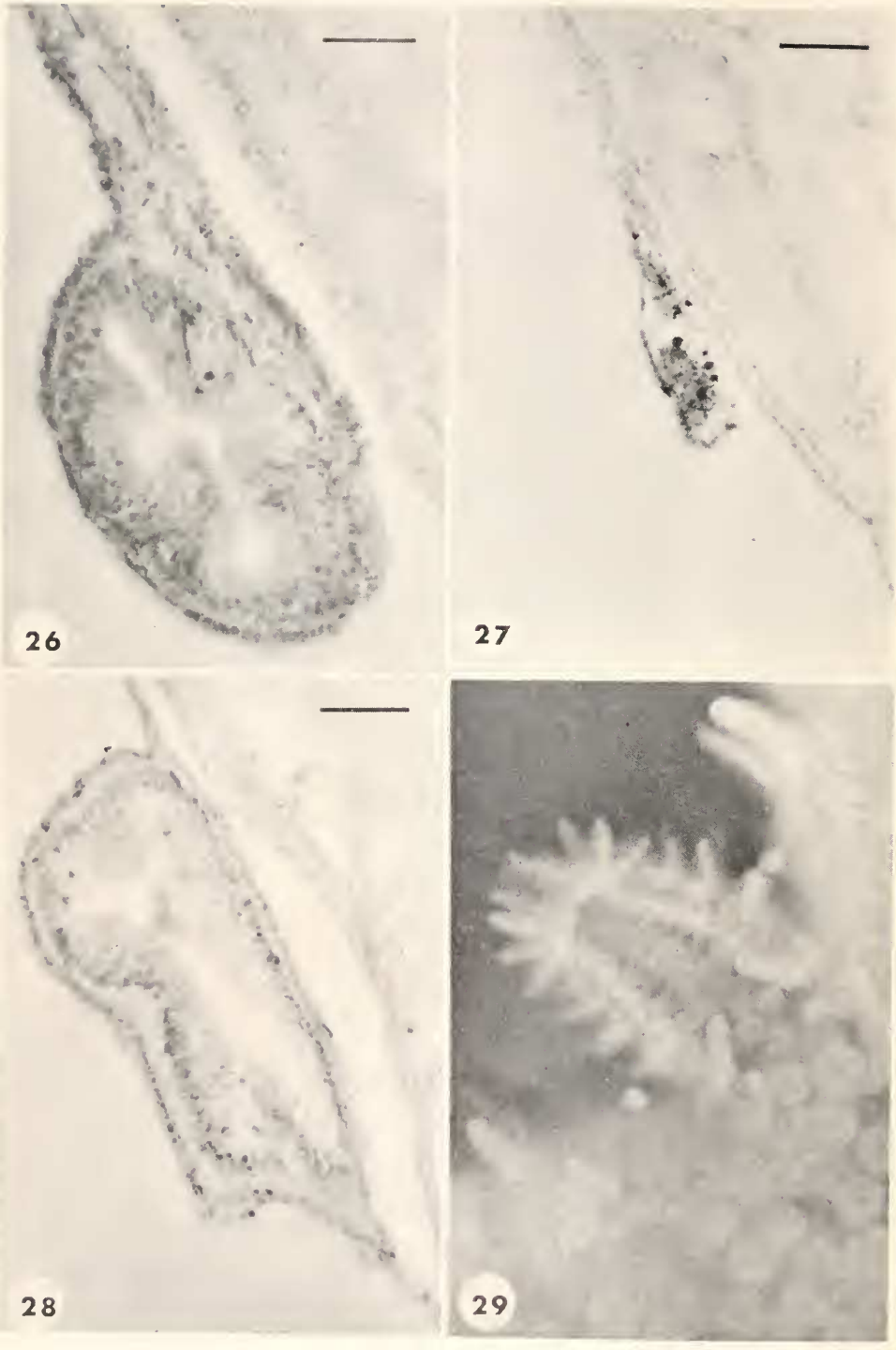
FIGURE 21. *Leptasterias:* section of the same regenerate about midway to its base. The wall is thin and consists almost entirely of the epithelium lining the tube, with abundant secretory cells. PTA hematoxylin. (Scale = 50 μ.)

FIGURE 22. *Leptasterias:* the same 5-week regenerate sectioned at its point of bifurcation. The arrow indicates the region in which the floor of the broad proximal duct rises to meet its roof and separate the two distal branches. Note thickenings of the lateral walls of the wider tube, shown here, marking incipient folds. PTA hematoxylin. (Scale = 100 μ.)

FIGURE 23. *Leptasterias:* cross-section of the 10½-week regenerate shown in Figure 18, at about midlength. Note the vertical depth of the tubes, and the histological differentiation between the side walls and the oral gutter. PTA hematoxylin. (Scale = 100 μ.)

FIGURE 24. *Leptasterias:* dissection of a specimen 6 weeks after removal of a single member of the pair of pyloric caeca in one ray. Compare the growth of this single regenerate with that shown in Figure 17, where both branches have been regenerating for 6 weeks. The arrow indicates the level of the section shown in Figure 25. (Approx. 6 ×.)

FIGURE 25. *Leptasterias:* cross-section of the distal end of the intact caecum shown in Figure 24 (arrow). The histology of this organ appears completely normal. PTA hematoxylin. (Scale = 50 μ.)



FIGURES 26–29.

and extended to join with those of the distal fragments, and tubular caecal regenerates had proceeded to grow from the pyloric duct, reaching a stage comparable with the 5-week organ shown in Figure 16 (but not attaining the level of the resorbing fragment).

Other experiments, involving simultaneous removal of all 5 pairs of pyloric caeca from specimens of *Pisaster* and *Asterias*, demonstrate that these animals rapidly recover from the effects of such radical surgery on the digestive tract and are capable of making good progress, within the time limits of the experiments, towards eventual replacement of all the missing organs. The events in regeneration of individual caeca after multiple excision are the same as those described for replacement of single pairs, but the rate of regeneration is considerably slower. Figure 15 shows one of the 5 pairs of regenerating caeca in a *Pisaster* specimen just short of 12 weeks following removal of all its caeca; regeneration in the remaining rays had proceeded to an equivalent condition. Regenerative progress here obviously is slower than that shown in the 12-week specimen illustrated in Figure 14 and appears about comparable with that of the 8-week single-pair regenerate shown in Figure 12. The rate of replacement in similarly operated specimens of *Asterias* is also reduced although characteristically more rapid than in *Pisaster*, as previously noted.

Sea-stars deprived of their pyloric caeca are incapable of digesting food until the caeca are replaced. This fact makes it possible to chart the return of function after total caecal extirpation, by observing the performance of specimens in repeated feeding trials. The following observations describe the feeding behavior of an operated *Pisaster* at the indicated post-operative intervals:

11 days:	offered food, ignores it
18 days:	offered food, ignores it
19 days:	attacks small mussel, later releases it unopened
3 weeks:	attacks and opens small mussel, remains humped over it several hours but produces no evidence of digestion
4 weeks:	repeats 3-week behavior, with same result
7 weeks:	opens small mussel, partially digests soft parts during several hours of feeding
11 weeks:	opens mussel, completely digests soft parts

FIGURE 26. *Asterias*, autoradiograph: cross-section of a two-week regenerate (see arrow in Figure 4) fixed three hours after injection of 0.5 μc. of thymidine-H^3, sectioned and prepared as described in the text. This is a normally growing regenerate, but it is evident that nuclear labeling is minimal or practically non-existent. Bouin, Harris hematoxylin. (Scale = 25 μ.)

FIGURE 27. *Asterias*, autoradiograph: two-week regenerate, section showing mesenteric tunnel distal to advancing tube. The specimen was fixed 6 hours after injection of 1.0 μc. of thymidine-H^3. Note the localization of labeled nuclei in the mesenchyme and peritoneum involved in tunnel formation. Bouin, Harris hematoxylin. (Scale = 50 μ.)

FIGURE 28. *Asterias*, autoradiograph: two-week regenerate, cross-section in proximal region of advancing tube; specimen fixed 12 hours after injection of 1.0 μc. of thymidine-H^3. Labeled nuclei appear in all cell layers of the regenerate but are particularly abundant in the lining epithelium where folds are developing. Bouin, Harris hematoxylin. (Scale = 50 μ.)

FIGURE 29. *Asterias:* oral aspect of a replacement ray known to have been formed within two weeks, following induced autotomy of the original. Approximately 15 pairs of tube feet have been produced, in addition to the terminal tentacle. This is the stage of regeneration represented in subsequent figures. (Approx. 10 ×.)

When this specimen was dissected within a few days of the last recorded observation, it proved to contain forked, tubular, somewhat folded regenerates in all rays, similar to those found in the specimen shown in Figure 15. In the 5 similar experiments performed, *Pisaster* specimens showed consistently that digestive functions were effectively re-established within 11 to 12 weeks. For comparison, the same experiments on *Asterias* indicated return of digestive function sometime between the fourth and sixth post-operative week. It may be noted as of passing interest that after a variable period of recovery all specimens acted "hungry" in the presence of food, and exhibited normal feeding behavior, for some time prior to the return of their ability actually to digest food enveloped by, or brought into, the cardiac stomach.

Turning now to the thymidine-H^3 experiments with *Asterias*, we may first note the technical point that 0.5 μc. total activity, available to the tissues for three hours, produces practically no nuclear labeling in the organism (Fig. 26). Doubling the total activity administered, and increasing the incubation time to 6 or 12 hours, brings about the abundant labeling shown in Figures 27 and 28. These figures illustrate clearly the very significant fact that thymidine incorporation, marking sites of impending or recent cell division, occurs at all levels of the two-week regenerate, from the elements of the far distal tunnel (Fig. 27) to the proximal outgrowth of the pyloric duct (Fig. 28). As these figures show, background activity is very low, and labeling of nuclei appears almost entirely limited to tissues involved in the regeneration of the caecum. The results of the entire series of experiments are consistent and demonstrate that mitotic proliferation proceeds very actively in the growing regenerate.

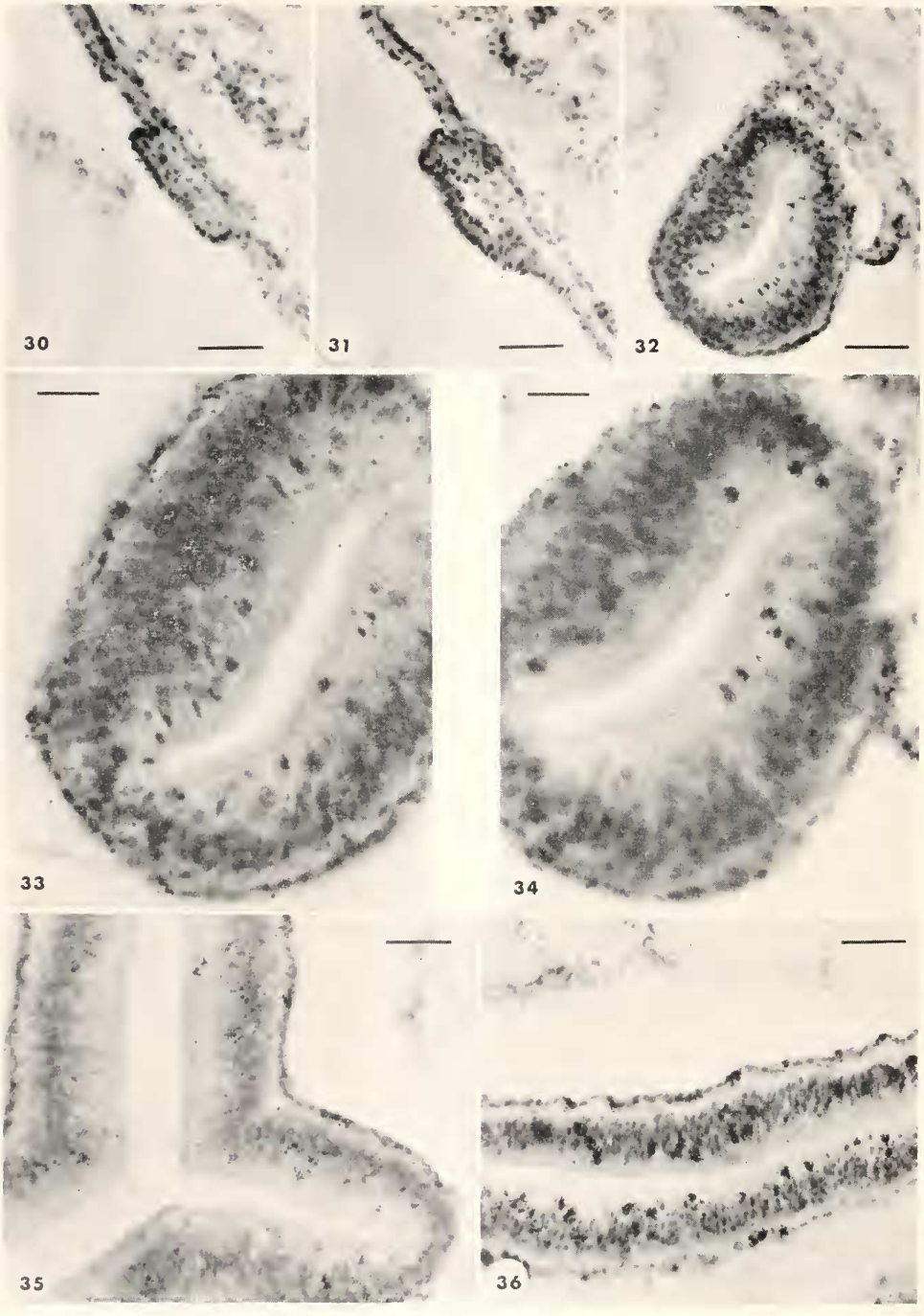
These observations are confirmed by the results of parallel experiments involving regeneration of pyloric caeca within regenerating rays. Figure 29 is an oral view of a two-week regenerate replacing an autotomized ray. The gradient of growth and differentiation in this young ray is clearly shown. Structures of the distal tip, while not yet of full size, are well differentiated; the most proximal features, such as tube feet, spines, and ossicles, are largest and oldest, and a gradient can be followed from the base of the new ray to a region just behind its tip, which contains the youngest structures and the zone of growth where they are formed. Sections of such a regenerating ray show that without question its visceral components are replaced through a sequence of events precisely comparable to those observed in the regeneration of excised pyloric caeca. Far distally, one encounters a low ridge-like structure (Fig. 30) which is continuous proximally with a typical mesenteric tunnel (Fig. 31). More proximally still, the summit of this tunnel is occupied by a tubular caecal outgrowth from the pyloric duct, and sections of such an outgrowth (Fig. 32) show that it is quite comparable to similar levels of previously observed replacement organs (Figs. 9, 13, for example). Further, as demonstrated in Figures 30, 31, and 32, the components of regenerating pyloric caeca within regenerating rays show the same autoradiographic evidence for mitotic activity as that found in material from the excised-caecum experiments.

But sections of regenerating caeca prepared for autoradiographs present not only the indirect evidence of thymidine uptake indicative of mitotic activity, but also conspicuous and unmistakable mitotic figures themselves. It will be recalled that this material was fixed in Bouin's fluid, while in earlier experiments all fixation

was in Helly's fluid. Restudy of sections of the Helly-fixed tissues reveals structures in the regenerating epithelium that can be interpreted as poorly-fixed mitotic nuclei; some of these may be identified in Figures 9, 11, 13, and 20, for example. In both the excised-caecum and whole-ray regenerates, particularly in *Asterias*, the advancing epithelial tube of the developing organ displays numbers of large, clear, often somewhat granular nuclei. These are especially numerous in the extreme distal regions of the tube, where most of the nuclei present appear to be of this type; proximal to the growing tip, in areas marked by differentiation of the epithelium into its specialized components, the cells with large nuclei gradually decrease in abundance, although remaining numerous and conspicuous. Here they lie at all levels in the epithelium, interspersed among the typical small, dense, basally crowded nuclei of the columnar cells lining the lumen; they are especially noticeable in the clear cytoplasmic zone above the nuclear band. It is apparently with the large nuclei that thymidine uptake in the epithelium is most frequently associated, particularly in the distal region of the regenerate, and the localization of unmistakable mitotic nuclei, as shown in Figures 32, 33, and 34, indicates that it is these cells, high in the epithelium, that are dividing most actively. It is evident that the majority of mitotic figures here are oriented parallel with the surface of the epithelium, and as a consequence new cells will be added in such a way as to contribute to the increasing diameter of the caecal tube behind the growing point.

Radioactivity in the epithelium is not altogether limited to the vesicular nuclei but is often found also in association with the small, dense nuclei of the differentiated cells. This is particularly evident in the older regions of the regenerate; note, for instance, the distribution of grains among the basal nuclei in the proximal section shown in Figure 35. There appears to be no zone in the epithelial lining of the two-week excised-caecum regenerate where mitotic activity can be said to occur with maximum intensity; the concentration of heavily labeled nuclei and of mitotic figures is greater a millimeter or two behind the solid tip of the outgrowth than in the tip itself, but it is noteworthy that cell division continues throughout the length of the tube, occurring even in the older, more proximal levels where differentiation has produced an essentially mature epithelium. As seen in Figures 28 and 35, evidence of mitotic activity is concentrated in the regions of the expanding caecum involved in the production of outfolding glandular pockets. The replacement caecum within a two-week whole-ray regenerate has not reached the stage of development characterized by wall folding, and as Figure 36 shows, mitotic activity in this tube seems fairly uniformly distributed in both older and younger regions of the lining.

Although directly observable mitotic figures are most conspicuous in the lining epithelium, cellular proliferation in the regenerate is by no means confined to this layer. As most clearly shown in Figures 35 and 36, thymidine uptake has occurred very actively in the peritoneal layer covering the outgrowing tube, and labeled nuclei are found also in the mesothelial constituents of the mesenteries, tunnels, and distal ridges. Here, as in the lining layer of the caecal tube, activity is most abundantly associated with large, clear nuclei and is less frequently found in the smaller, more irregularly shaped nuclei of typical cells of the peritoneum. The occurrence of labeling in the mesenchyme is less obvious; some activity is apparent among the mesenchyme cells forming the core of the far distal ridge in Figure 30, and perhaps



FIGURES 30–36.

in the tunnel shown in Figure 31. The resolution of the radiographs being what it is, however, it is impossible to state with confidence whether the developed grains scattered in the region between the lining epithelium and the covering peritoneum, at more proximal levels, actually pertain to nuclei of the mesenchymal cells, which are spread very thinly here. As the figures indicate, these grains almost always appear near heavily labeled nuclei of the peritoneum.

Discussion

No significant differences appear when events in caecal regeneration are compared among the three asteriids studied. The rate differences encountered in the different experimental series are almost certainly expressions of temperature effects. According to station records (D. P. Abbott, personal communication), mean monthly sea-water temperatures at the Hopkins Marine Station for the period occupied by the regeneration experiments on *Leptasterias* and *Pisaster* ranged between 14 and 16° C. In contrast, mean surface water temperatures at Woods Hole during the months covering the *Asterias* experiments averaged about 20° C. (D. Bumpus, WHOI sheets). It is thus not surprising that regeneration in *Asterias* should be found to proceed so much more rapidly than in the other asteriids studied. The rate in *Henricia* (Anderson, 1962) is comparable with that in the other West Coast forms, producing nothing more advanced than simple tubular regenerates in the 8-week period covered by the experiments. The peculiar involvement of the peritoneum in *Henricia,* lacking in asteriids, has already been mentioned. Beyond this, however, one may conclude that the asteriid experiments, carried to the point at which essentially complete, normal, and functional organs have been produced, reveal the kinds of changes that could be expected if the brief series of experiments on *Henricia* were extended to cover the later stages of regeneration. One would expect, of course, that some particular development in

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These figures are autoradiographs of sections showing regeneration of pyloric caeca within regenerating rays like that in Figure 29. Specimens were fixed 12 hours after injection of 1.0 μc. of thymidine-H^3 and prepared as described in the text.

Figure 30. *Asterias:* cross-section, far distally in the ray, showing localization of labeled nuclei in mesenchyme and peritoneum of a ridge forming in advance of the mesenteric tunnel. Bouin, Harris hematoxylin. (Scale = 25 μ.)

Figure 31. *Asterias:* same specimen, more proximally. The mesenteric tunnel has formed at this level and shows abundant labeled nuclei and mitotic figures, particularly in the peritoneum. Bouin, Harris hematoxylin. (Scale = 25 μ.)

Figure 32. *Asterias:* same specimen, more proximally still; cross-section of tubular regenerate. Labeled nuclei and mitotic figures are apparent in the peritoneum as well as in the lining epithelium. Compare Figures 9, 13. Bouin, Harris hematoxylin. (Scale = 25 μ.)

Figures 33 and 34. *Asterias:* same specimen, sections similar to the last, in detail. The epithelium lining the regenerate is well differentiated; large nuclei high in the epithelium are mitotically active; labeled nuclei are numerous also in the peritoneum. Bouin, Harris hematoxylin. (Scale = 10 μ in both figures.)

Figure 35. *Asterias:* same specimen, sectioned more proximally where the tubular regenerate has developed folded walls. Note that labeled nuclei are scattered in all layers, apparently including the subepithelial mesenchyme. Bouin, Harris hematoxylin. (Scale = 25 μ.)

Figure 36. *Asterias:* longitudinal section of the tubular regenerate in a similar specimen to the last, similarly prepared. Heavily labeled nuclei in all layers, relatively uniformly distributed through the length of the tube, indicate that cellular proliferation is not limited to the advancing tip. Bouin, Harris hematoxylin. (Scale = 25 μ.)

the floor of the regenerate would eventually lead to the formation of Tiedemann's pouch, with its specifically oriented flagellary channels—a structure not produced in the other forms. Such changes could be interpreted as simply an extension of the process of cell localization observed in all the other species, as a result of which the secretory cells become segregated in the incipient glandular pouches and the more generalized current-producing cells come to be concentrated in the roof and floor of the median duct. In *Henricia* the final disposition of glandular cells is somewhat different (Anderson, 1960).

Studying the regenerative process in all the species observed, whether the new pyloric caecum is replacing an operatively-removed organ or is growing in connection with the regeneration of an autotomized ray, one can only be struck by the aptness of Hyman's simple statement (1955, p. 314): "The pyloric caeca are replaced by outgrowth from the old ones. . . ." In all cases, after a period of reorganization, whatever remains as a stump of the original organ produces an outgrowth that always appears as a simple epithelial tube, continuous with its own lining layer, which secondarily bifurcates and forms pockets. Hyman's statement continues to the effect that regeneration occurs in the same manner as post-larval growth; with this in mind, some descriptions of the events involved in the original formation of the caeca will be of interest, for purposes of comparison. Cuénot (1887), for example, refers briefly (p. 33) to the development of the pyloric caeca, pointing out that morphologically they are extensions of the gastric sac (*sac stomacal*); he gives a drawing of a developing caecum from a young *Astropecten* (Plate III, fig. 5), describing this as a tubular projection from the gastric sac without definite folds but with the same histology as that of its parent organ. Gemmill (1912), describing the development of *Solaster endeca*, gives a full account of the origins and relationships of the pyloric caeca (p. 40): "The paired radial diverticula appear as folds on the roof of the stomach which converge towards a central point. . . . The outer extremities of the folds . . . become elevated from the surface and grow out as blind pouches into the rays. . . . During the fourth month the radial diverticula begin to broaden at their outer ends. This is followed by bifurcation at these ends, with accompanying division of their suspensory mesenteries and of the pockets of epigastric coelom contained therein." Other accounts, notably those of MacBride (1896, 1914), provide generally comparable descriptions.

The similarity between embryonic or post-larval development, as outlined in the foregoing passages, and the events involved in regeneration of the organs is clearly evident in the account by Yamazi (1950) of regeneration after autotomy in the fissiparous, multirayed sea-star *Coscinasterias acutispina*. In this species the normal number of rays is 8; full-grown individuals divide across the disk, and each daughter individual then grows four small replacement rays. The pyloric stomach repairs itself, gives off a rectal sac, grows to the edge of the disk, and produces a small, tubular outgrowth leading into each of the four new rays. As Yamazi describes further changes (p. 182): "The pyloric caecum soon furcates into two hollow canals at the position of the mesenteries placed on the median line of the ray cavity. The stalk of these branches remains as a common duct. . . . The branches which are originally simple canals, issue laterally into two series of short lobular branchlets. Meanwhile, the median canal becomes flattened laterally."

The events thus described, and the structural features and changes illustrated in Yamazi's drawings, are obviously very closely similar to post-larval development as described by others; they also resemble very markedly the kinds of regenerative changes found in the present studies of asteriids.

It will be noted that Yamazi's statement refers briefly to a relationship between caecal outgrowths and the positions of the mesenteries in regeneration, and Gemmill's description of post-larval growth, quoted above, indicates that such a relationship characterizes the original formation of the organs also. On this subject Gemmill has more to say (p. 34): "As the paired diverticula of the enteron grow out, pockets from the epigastric coelom extend outwards along their aboral aspect, and these pockets bifurcate as the diverticula themselves become divided into two . . . the paired radial extensions of the mesentery . . . do not become broken up into fibers, but remain as the boundary-walls of the epigastric coelomic pockets, which in the adult lie above the paired radial diverticula of the stomach." As a result of the present studies, and those reported earlier on *Henricia,* we are now in a position to understand that in caecal regeneration the paired mesenteries do not simply *accompany* the outgrowing regenerate. Rather, the positioning and bifurcation of the mesenteric tunnels (and of the pockets of epigastric coelom which they enclose) are events that occur in advance of the outgrowth of the caecal tube in each case. In regeneration of excised caeca, it is easy to visualize how the mesenteric tunnels form through the zippering-up, so to speak, of the remnants of the pre-existent paired mesenteries. What is perhaps more intriguing is the fact that in regeneration of an entire ray the mesenteric tunnels must form anew, traversing the newly formed aboral body wall, as in the original growth of the ray. The future mesenteric tunnel is represented far distally in the new ray by the low ridge which will later become elevated and contain a coelomic extension. Arising in this way, the tunnel must bifurcate at the appropriate level so as to guide the caecal tube in its outgrowth, and in its subsequent broadening and bifurcation. What it is that guides the formation of the tunnel is not apparent.

The partial-extirpation experiments on *Leptasterias* contribute to a demonstration that caecal outgrowth, even with completely re-formed guiding mesenteries in place, occurs only in an outward direction, and only from centrally connected remnants or stumps of excised organs. They show also that remnants of an intact caecum, so long as they are centrally attached, are not noticeably utilized as a source of materials for the replacement of missing portions. Distally isolated fragments are resorbed without exerting any demonstrable effect, inhibitory or otherwise, on the progress of regeneration from the central stump. The limited series of experiments does not, however, provide an illustration of what interaction might occur when a centrally attached caecal outgrowth, advancing in its guiding mesentery, encounters a resorbing, distally isolated fragment.

The ability of *Pisaster* and *Asterias* (and presumably other species as well) to replace all pyloric caeca simultaneously after total extirpation is noteworthy. Excision of all the caeca removes the only recognized source of digestive enzymes and thus precludes the possibility of effective feeding until functional organs can be regenerated. The observations on post-operative feeding behavior of caecumless specimens confirm the supposition (previously advanced for *Patiria;* see Anderson, 1959) that the caeca are indispensable for the digestive process. At the same time,

removal of the caeca eliminates the major storage organs for nutritional reserves, against which the animal would normally draw when for any reason feeding is not taking place. The energy sources for maintenance of metabolism during the period of enforced starvation pending caecum regeneration have not been identified. One can only assume that, in view of the invariably successful and relatively rapid replacement of the excised organs, subsidiary stores in the body wall and elsewhere must provide adequate reserves.

As previously noted (Anderson, 1962), growth patterns in regenerating pyloric caeca are different from those in the surrounding body wall. Yamazi's work (1950) on regeneration in *Coscinasterias* provides a graphic illustration (see his Figure 4, page 183) of the precociously developed distal structures in the body wall, advancing ahead of the zone of growth which establishes the age-gradient between itself and the base of the ray. Yamazi shows, and the present study of asteriids confirms with additional details, that there is no comparable differentiated region at the tip of the outgrowing caecum. Histologically, the tip of the caecum is the simplest and youngest region; it is noteworthy, however, that differentiation of the lining epithelium begins almost immediately behind the advancing tip, with the production of secretory cells and the introduction of specialized characteristics of the epithelial cells.

In view of the conclusive evidence, both direct and indirect, revealing the intensity of mitotic activity in the regenerating pyloric caecum of *Asterias*, it is no longer necessary or valid to invoke mobilization of amoebocytes as a primary source of new cells for the replacement organ (*cf.* Anderson, 1962). Failure to recognize mitotic figures in the regenerating caecum of *Henricia* can be attributed chiefly to a technical deficiency, involving consistent use of a fixative (Helly's) chosen as ideal for cytoplasmic features but providing poor preservation of nuclear details. Also relevant is the fact that regeneration in *Henricia* is relatively slow, with mitotic activity much less prevalent than in the rapidly growing regenerates in *Asterias*. There can be little question, however, that many scattered structures in sections of *Henricia* material, originally interpreted as pycnotic nuclei, actually represent collapsed mitotic figures. Having demonstrated the widespread occurrence of cellular proliferation in *Asterias*, one has no reason to doubt that regeneration in *Henricia*, so closely similar in all other respects, must involve the same source of cells for incorporation into the developing organ.

With a heretofore puzzling question disposed of, visceral regeneration in the asteroids studied can now be compared more meaningfully with the only other case of gut-replacement in echinoderms that has been similarly investigated—that of regeneration following evisceration in holothuroids. Dawbin (1949) has shown that in *Stichopus*, beginning about 40 days after loss of the gut, mitotic activity becomes widespread in the layer of cells lining the newly developed lumen of the regenerating alimentary tract. These cells, it will be noted, form a solid cord of mesenchyme at the thickened mesentery edge, and it is in this cord that an irregular lumen gradually forms. The cells dispose themselves so as to constitute a lining for the tube, and through continuing division provide for the growth of the gut until it eventually reaches normal size. As Dawbin points out, in order to accommodate the ever-increasing diameter of the tube the covering peritoneum must, and does, add cells also; but most of the mitotic activity observed is in the

cells of the gut lining. Interestingly, cell division in the regenerating caecum of *Asterias* is observed in these same two layers, and its occurrence in the intervening thin layer of mesenchyme is questionable at best on present evidence. One point of contrast deserves emphasis: in asteroids, the cell layer lining the lumen of the regenerating caecum is always continuous with the epithelium of the pyloric duct and, as we have seen, extends outward by proliferation of cells belonging to this layer. In *Stichopus,* the lining proliferates and differentiates in place, from mesenchymal accumulations, without reference to or connection with the corresponding layer of the esophagus remnant lying anteriorly (= orally) in the supporting mesentery. In this respect, of course (see discussion in Anderson, 1962), gut regeneration in *Stichopus* differs even from that in other sea-cucumbers studied (*Holothuria* and *Thyone*), where the continuity of layers is similar to that found in asteroids. The existence of these differences makes it appear that a re-study of events in visceral regeneration in holothuroids, with particular attention to histological details, localization of mitotic activity and cellular differentiation, and related matters, would provide a valuable contribution for comparative purposes.

The thymidine-H^3 experiments on *Asterias* were originally designed, in the absence of recognizable mitotic figures in material that had been studied, simply to provide evidence for the occurrence or non-occurrence of cell division in the regenerating caecum. They were not precise enough or extensive enough to serve as a basis for detailed analysis of cell-population dynamics in this system, and such an analysis has not been attempted. However, with the original objective now attained, with the added bonus of confirmation from directly observed mitotic activity in the same sections, we may note briefly some additional information provided by study of the autoradiographic preparations. The appearance of the radioactive label in such profusion throughout the tissues of the regenerating caecum, after administration simply by injection into the coelomic fluid, provides a further indication of the activity of metabolic exchange between the circulating fluid and the pyloric caeca, as already demonstrated for various nutrients by Ferguson (1964a, 1964b).

Considering the difference in general metabolic levels, the duration of the mitotic cycle in *Asterias* is undoubtedly much greater than the 40 or so hours given by Lajtha (1957) for human bone-marrow cells in culture, or the approximately 20 hours suggested as a reasonable average by Quastler and Sherman (1959) for proliferating cells in the duodenal lining of the mouse. Thus, given the 6- to 12-hour incubation time in the present experiments, it is highly unlikely that any cell in the system can have proceeded through more than one mitotic cycle in the presence of the radioactive label. Heavily-labeled nuclei must have passed through a significant fraction of their premitotic synthetic phase during the period of incubation; more lightly labeled nuclei must represent cells exposed hardly at all to thymidine-H^3, or immediately post-mitotic daughter cells carrying only a part of the label taken up by the parent nucleus. In some cases, particularly those involving 12-hour incubation periods, grain-density is so extreme over many nuclei that the mitotic status of the cells cannot be determined (*cf.* Fig. 36). Where labeled nuclei can be identified as premitotic, it has already been pointed out that they are most frequently of the large, vesicular variety with which mitotic activity seems usually to be associated, in both lining epithelium and peritoneum. These probably

represent undifferentiated cells; their progeny, it would appear, either remain undifferentiated and furnish the stock for continuing divisions; or else, in the lining layer, elongate, establish contact with the basement membrane, and transform into the columnar cells typical of the developing epithelium. The fact that some activity is associated with small, dense nuclei characteristic of these differentiated cells may identify them as post-mitotic types retaining label incorporated by a parent nucleus; alternatively, it may indicate that even differentiated cells can synthesize DNA and continue to proliferate. Leblond and Messier (1958) have reported encountering copious labeling of the nuclei of goblet cells in the intestinal epithelium of mice, under experimental conditions indicating that this is a premitotic phenomenon rather than a post-mitotic inheritance from a parent cell.

It seems useless to attempt further analysis of these results until they can be extended and refined by additional experiments. It is suggested, however, that the regenerating pyloric caecum of asteroids, like the regenerating digestive system of holothuroids, provides excellent material for the study of cell proliferation and differentiation. The events in regeneration of the parietal parts of an autotomized ray, and the distribution of mitotic activity in regions other than those directly involved in caecal regeneration, are related subjects best reserved for discussion elsewhere.

Summary

1. The regenerative replacement of excised pyloric caeca has been studied in three species of sea-stars belonging to the Family Asteriidae. Regenerating specimens have been observed, and the histological events of regeneration followed by serial sections, to the point at which essentially normal organs are again in place and functioning. The process of regeneration is practically identical in all, but it occurs at a much slower rate in *Leptasterias* and *Pisaster* than in *Asterias*. Rate differences are attributable to differences in environmental temperature.

2. Caecal regeneration involves a sequence of changes resembling those previously observed in *Henricia*. Mesenteric tunnels guide the advance of simple tubular outgrowths from the pyloric duct; bifurcation of the caecum occurs at the point where the tunnel forks, giving rise to paired tubes growing distally parallel with each other. Differentiation of epithelial elements, with functional secretory cells, proceeds close behind the advancing tip of each tube. More proximally, lateral expansions produce pockets in which gland cells become localized, while less highly-specialized current-producing cells line the roof and floor of the median duct. The gradient of differentiation extends from the young, undifferentiated growing tip to the oldest region at the base of the regenerate. The pyloric caecum growing inside a regenerating ray following induced autotomy forms through the same sequence of events found in replacement of an excised caecum. Comparison with descriptions of post-larval formation of the pyloric caeca in the developing sea-star reveals that regeneration follows the same course as original formation.

3. Isolated segments of partially extirpated organs are resorbed without producing regenerative growth; their presence in the mesenteric tunnel does not affect normal outgrowth from the central stump. *Pisaster* and *Asterias* are capable of regenerating all 5 pairs of pyloric caeca at once, at rates somewhat slower than those involved in single-pair replacement. The return of function in these caecum-

less animals has been charted by observation of feeding behavior and digestive capability.

4. The suggestion that growth of the replacement caecum occurs through mobilization and incorporation of amoebocytes, based on earlier failure to identify mitotic activity in *Henricia,* is now shown to be invalid. Autoradiographs made from sections of two-week regenerates in *Asterias* after injection of thymidine-H^3 reveal large numbers of cells in some phase of mitotic activity. Direct observation of mitotic figures in these same preparations confirms the fact that growth of the regenerating caecum involves mitotic proliferation. Cell division is most common among apparently undifferentiated cells with large, vesicular nuclei; it occurs where such cells occur, in both lining epithelium and covering peritoneum, at all levels of the advancing regenerate. In proximal regions, dividing cells are most numerous where the caecal walls are outfolding to form glandular pockets.

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